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**The impact of isoniazid preventative therapy and antiretroviral therapy on tuberculosis (TB) in HIV-infected children in a high TB incidence setting.**

Thesis presented for the degree of

**Masters of Medicine**

by

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School of Child and Adolescent Health

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University of Cape Town

April 2011

**Supervisor**

Professor Heather J. Zar

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## **Declaration**

I, Lisa Jane Frigati, present this thesis in fulfilment of the requirements for the degree of Masters of Medicine in the Department of Paediatrics, School of Child and Adolescent Health, Faculty of Health Sciences, University of Cape Town. The contents of this thesis are entirely the work of the candidate.

The candidate was one of the study doctors on the 'Strategies for prevention of opportunistic infections in HIV-infected South African Children: Comparison of 2 Trimethoprim - Sulphamethoxazole prophylaxis regimens with and without concomitant Isoniazid - impact on morbidity, mortality, bacterial resistance and incidence on tuberculosis study - a randomized controlled trial' for 6 months in 2007 before commencing her registrar training. The analysis plan was developed by the candidate during her registrar training with input from an epidemiologist with training in statistics (K. Kranzer - 2<sup>nd</sup> author). The planned analysis was then reviewed by the statistician that worked on the original randomized controlled trial (Carl Lombard - 4<sup>th</sup> author) and after this input a separate database was constructed by the candidate and K. Kranzer. K. Kranzer performed the analysis. The original manuscript also contained a case control study which has been omitted from the final manuscript. The manuscript was written and edited by the student with input and comments from co-authors including the supervisor and collated to form the final manuscript.

The work on which this thesis is based is original research and has not, in whole or in part, been submitted by myself or by any other person for another degree at this or any other university.

Signature and date:

## **Acknowledgements**

I would like to acknowledge all the co-authors for their contributions as well as all the study doctors, nurses and patients as well as their caregivers.

I would especially like to acknowledge Dr Kranzer and Prof Zar for all their input.

University of Cape Town

## **Abbreviations**

**ART** - antiretroviral therapy

**BCG** - Bacille Calmette-Guérin

**CXR** - chest radiograph

**IPT** - INH preventative therapy

**HIV** - human immunodeficiency virus

**MDR** - multidrug resistant

**PMTCT** - prevention of mother to child transmission

**PCR** - polymerase chain reaction

**SMX** - sulfamethoxazole

**TB** - tuberculosis

**TMP** - trimethoprim

**TST** - tuberculin skin test

**TU** - tuberculin unit

**WHO** - World Health Organization

## **PART A: PROTOCOL**

### **CV of the supervisor**

#### **BIOGRAPHICAL SKETCH**

NAME		POSITION TITLE	
Zar, Heather Joy		Professor, School of Child and Adolescent Health, University of Cape Town	
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of Witwatersrand, South Africa	MBBCh	1980-85	Medicine
Albert Einstein College of Medicine, LIJ Medical Center	Residency	1988-91	Pediatrics
Columbia University	Fellowship	1991-94	Pediatric Pulmonology
University of Cape Town	PhD	1998-00	Pediatric HIV-associated lung disease

#### **A. Position and Honors. Positions and Employment (last 5 yrs)**

- 2008: Professor and Chair School of Child and Adolescent Health, University of Cape Town
- 2007: Professor, University of Cape Town, School of Child and Adolescent Health
- 2007: Elected a Fellow of the University of Cape Town, South Africa
- 2006: Head of Department of Paediatric Pulmonology, School of Child and Adolescent Health, Red Cross Children's Hospital, University Cape Town
- 2002: Associate Professor, University of Cape Town, School of Child and Adolescent Health

#### **Honors / awards (last 5 yrs)**

- 2005: University of Cape Town Research award
- 2005: Best Senior Investigator, South African Thoracic Society
- 2005: Aspen Pharmacare Award from South African Thoracic Society

- 2005: Astra-Zeneca Research Award from South African Thoracic Society
- 2006: Finalist Department of Science, South Africa – Distinguished Woman in Science Award
- 2006: South African National Research Foundation B2 rated researcher as an internationally acclaimed researcher
- 2007: Fellow University of Cape Town
- 2009: Best publication award, South African Thoracic Society
- 2010: Special award from the International Pediatric Pulmonology Congress for “outstanding leadership and distinguished service to the children with the greatest need”

## B. Selected peer-reviewed publications (in chronological order).

(Publications selected from over 100 peer-reviewed publications)

1. **Zar HJ**, Brown G, Donson H, Brathwaite N, Mann MD, Weinberg EG. Home made spacer devices for bronchodilator therapy in children with acute asthma – a randomized trial. *Lancet* 1999;354:979-82
2. **Zar HJ**, Weinberg EG, Binns HJ, Gallie F, Mann MD. Lung deposition of aerosol – a comparison of different spacers. *Arch Dis Child* 2000;82:495-498.
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- chest radiography in detecting mediastinal lymphadenopathy in suspected pulmonary tuberculosis. *Arch Dis Child* 2005;90:1153-56
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### Ongoing Research Support

1. National Institute of Health, USA, RO1, Zar (PI), 2008-2013  
Role: PI, Diagnosis of Tuberculosis in HIV-infected children – development of improved microbiological and immunological strategies RO1 HD058971-01
2. National Research Foundation, South Africa, Zar (PI), 01/01/2008-12/31/2011  
Incentive funding for rated researcher
3. National Research Foundation, South Africa, Zar (PI), 01/01/2007-12/31/10  
Role: PI, Molecular diagnosis of Pneumocystis pneumonia and emergence of resistance
4. Dept of Health, Research Program of Operational Plan for Comprehensive HIV Care, South Africa., Zar (PI), 01/06/2007–01/06/2011  
Role: PI, Strategies for prevention of opportunistic infections in HIV-infected South African children: comparison of 2 cotrimoxazole regimens with and without concomitant isoniazid
5. Medical Research Council, Zar (PI), 01/01/2006-12/31/09  
Role: PI, Long term study of 2 isoniazid (INH) prophylactic regimens with concomitant cotrimoxazole (CTX) in HIV-infected children – impact on morbidity, mortality, bacterial resistance and incidence of tuberculosis
6. Wellcome Trust Strategic award WT084323MA, Wilkinson (PI), 2008-2013  
Role: co-investigator, A centre for clinical infectious diseases research at the University of Cape Town
7. Global Alliance for Vaccines and Immunization (GAVI), 01/10/2009-1/10/12  
Role: site PI, Case-control study on effectiveness of pneumococcal conjugate vaccine against pneumonia in HIV-infected and HIV non-infected children in South Africa.
8. National Institute of Health, USA, 01/9/09-1/9/2014  
Role: co-investigator, Tuberculosis Clinical Diagnostics Research Consortium. RFP-NIAID-NIH-AI2008026
9. European & Development Countries Clinical Trial Partnership (EDCTP), 2010-2013

- Role: Paediatric PI, Evaluation of multiple novel and emerging technologies for TB diagnosis, in smear-negative and HIV-infected persons, in high burden countries (the TB-NEAT study)".
10. National Institute of Health, USA, 01/9/11-2013  
Role: site PI, Optimal dosing of first line antituberculosis and antiretroviral drugs in children. R01 HD069175-01
  11. Bill and Melinda Gates Foundation, 2010-2016  
Role: PI, the Drakenstein Child Lung Health Study.

University of Cape Town

## **Protocol**

### **The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis (TB) in HIV-infected children in a high TB incidence setting.**

Protocol version 5, November 2010

**MMed Student:** Lisa Jane Frigati

**Supervisor:** Heather Zar

## **Study Summary**

Tuberculosis (TB) is an important cause of morbidity and mortality in children.<sup>1 2</sup> The incidence and severity of childhood TB is increased by HIV infection.<sup>3</sup> HIV-infected children with TB infection are at risk of more rapid progression to disease, more severe disease and increased mortality.<sup>4-6</sup> The Western Cape in South Africa has an extremely high incidence of TB especially among HIV-infected infants.<sup>3</sup>

There are relatively few interventions to decrease the risk of childhood TB in HIV-infected children. Adult studies have shown that INH preventative therapy (IPT) decreases the risk TB in HIV-infected adults on ART.<sup>7 8</sup> There are currently no such studies in children although it has been shown that IPT and ART independently decrease the risk of tuberculosis in children.<sup>9-12</sup>

The aim of this study is to investigate the combined effect of IPT and ART on TB incidence in HIV-infected children. A cohort analysis will be performed using data from a prospective, double-blinded placebo controlled trial of INH versus placebo in HIV-infected children in Cape Town, South Africa.<sup>9</sup> Ethical approval for this study was obtained from the Research and Ethics committees of Faculty of Health Sciences of the Universities of Cape Town and Stellenbosch, South Africa (Rec: 057/2002).

## **Background**

In Cape Town, South Africa, the incidence of TB in children has been reported to be approximately 407/100 000 population and to account for around 13.7% of the total TB burden.<sup>13</sup> This burden of TB was 20 times higher among HIV-infected than uninfected children - with 23 active cases of TB seen per 100 HIV-infected children compared to 7.2 in HIV uninfected.<sup>14</sup>

HIV-infected children are at increased risk of developing TB due to a higher burden of exposure to TB infected cases and impaired immunity.<sup>15</sup> HIV-infected children with severe immuno-suppression (CD4 percentage < 15% and viral load > 100 000) are at a 4 times increased risk (12.4% versus 3.3 % in children with a CD4 count greater or equal to 15%) of progression to TB disease over 3 years.<sup>16</sup>

Bacille Callmette Guerin (BCG) vaccination, IPT and ART are possible strategies to prevent TB in HIV-infected children. World Health Organization (WHO) guidelines have recently been revised to advise against BCG vaccination in HIV-infected infants due to the high risk of disseminated BCG and associated mortality.<sup>17</sup>

IPT is part of standard guidelines to prevent TB in HIV-infected and uninfected children who have household exposure to adult TB. Adult studies have shown that IPT decreases TB incidence in HIV-infected adults with a positive tuberculin skin test (TST), however there are few studies in children. IPT is rarely rolled out in resource limited countries where access to screening tools for TB is limited.<sup>18</sup> It is important to accurately exclude TB disease before starting IPT otherwise there is the risk of using mono-therapy and the possibility of drug resistant TB developing.

The use of ART has also been shown to decrease the incidence of TB in children. The incidence of TB in children on ART versus those not on ART is 6.4 cases per 100 patient years versus 53.3 cases /100 patient years (OR 16.6- 95% CI 12.5-22.4).<sup>10</sup> Studies in Brazil and South Africa have shown that ART reduces the incidence of TB by around 70 to 80%.<sup>8 19</sup> However, the risk of TB still remains higher than that in HIV-uninfected children.

Observational studies suggest that INH preventive therapy is protective in adults receiving ART.<sup>8 19</sup> Several randomized trials investigating the combined effect of INH prophylaxis and ART are underway in adults but data in children are scarce. The objective of this study will be to investigate the combined effect of INH prophylaxis and ART on TB incidence in HIV-infected children living in a high TB incidence setting.

## **Study Description**

### **Objective**

To investigate the combined effect of INH prophylaxis and ART on TB incidence in HIV-infected children.

### **Study Design**

Retrospective analysis of data that was obtained in a prospective cohort study will be carried out to investigate the effect of INH and ART on TB incidence. Individuals will be censored at death, date of TB diagnosis, loss to follow up or on 31 December 2007 (the time at which INH prophylaxis was discontinued in the study cohort). Individuals may contribute time to four groups: time on placebo, time on INH only, time on ART only, time on ART and INH.

Confounding variables to be investigated include

- (At enrolment)
- Nutritional status
- CD4 percentage / count
- Age
- Gender
- Cotrimoxazole
- Prior TB

### **Study Site**

The original study was done at Red Cross War Memorial Children's Hospital and Tygerberg Children's Hospital, both situated in Cape Town, South Africa.

### **Study Population**

The study population comprised HIV-infected children older than 8 weeks attending Red Cross Children's Hospital, University of Cape Town or Tygerberg Children's Hospital, Stellenbosch University.

### *Inclusion Criteria*

- Weight greater than 2.5kg

- Ability to attend regular follow-up visits

#### *Exclusion criteria*

- Current use or need for INH prophylaxis
- Prior hypersensitivity to sulphur drugs or INH
- Severe anaemia (haemoglobin < 70 g /l)
- Neutropaenia (absolute neutrophil count < 400 cells /ul)
- Non-reversible renal failure

TB disease at the time of enrolment was not an exclusion criterion. Children completed TB therapy according to national guidelines and thereafter were randomized to INH or placebo.

#### *Follow-up*

As part of the original study children were evaluated every four weeks for the first six months, every six weeks for the second six months and thereafter every two to three months depending on medical and social circumstances. Clinical and historical data were collected at each visit. A tuberculin skin test (TST, 2 TU RT23, Statens Serum Institute, Copenhagen, Denmark), and chest radiograph (CXR) were done at 6 monthly intervals. A child exposed to a TB source (case), hospitalized for respiratory illness, or developing symptoms of TB at any time during the study period was investigated for TB infection and disease. For pulmonary TB this included a TST, a chest radiograph and two induced sputum specimens for acid fast staining and *Mycobacterium tuberculosis* (*M. tuberculosis*) culture. Additional specimens were submitted as clinically indicated. Children exposed to a household TB case were given INH prophylaxis according to standard guidelines and then returned to their initial randomization protocol without being un-blinded.

#### *INH*

Enrolment began in January 2003. Children enrolled before May 2004 were randomly allocated to receive INH (10 mg/kg) or placebo. All children receiving placebo were switched to INH prophylaxis in May 2004 on the advice of the Data Safety and Monitoring Board (DSMB) due to the decreased mortality observed in children on INH.<sup>9</sup> All children enrolled thereafter received INH. INH was stopped in all children in December 2007 on the advice of the DSMB as most children were then on ART and had taken INH for a prolonged period.

### *ART*

ART was not widely available at the start of the study but was obtained for some children through the participation in pharmaceutical trials or charitable donations. Standard guidelines for ART in children were developed after the establishment of the national ART programme in 2004. Thereafter increasing numbers of children initiated therapy according to medical and social criteria described in the national guidelines.

### *Diagnosis of TB*

Cases of TB were classified as definite or probable according to clinical, radiological and microbiological findings as follows:

Definite TB: culture positive for M tuberculosis on a sputum or other sample

Probable TB: CXR suggestive of TB (lymphadenopathy, miliary pattern, pleural effusion, bronchial compression or parenchymal infiltrate) plus at least one of the following: a positive TST, a history of a close contact with an adult with TB, loss of weight or failure to gain weight within the previous 3 months, or a positive smear microscopy for acid fast bacilli on sputum.

Chest radiographs were reported by a single radiologist, blinded to the diagnosis of TB, according to a standardized format. Probable cases were reviewed by an independent experienced paediatrician blinded as to whether the child was on INH, placebo and/or ART.

## **Methods**

### *Data Management*

All data being used in the cohort analysis will be extracted from pre-existing databases.

### *Proposed analysis*

The analysis will be conducted using Stata (version 10.1, College Station, Texas) Descriptive statistics of the baseline data and follow-up data of the cohort will be calculated as medians, 25th and 75th percentiles and frequencies.

Cox proportional hazards regression will be performed using time since randomization as the time scale. A univariate analysis will be conducted using immunodeficiency, anthropometric z-scores and age at enrolment, gender, prior TB, cotrimoxazole daily or three times per week, placebo, INH, ART and INH and ART.



Age and immunodeficiency at enrolment will be included a priori in the multivariate model. Possible other confounders including WHO clinical staging and nutritional status at enrolment and prior TB, gender and cotrimoxazole will be assessed with regards to their impact on the effect estimates. Variables acting as confounders will be included in the final model.

## **Ethical Considerations**

### *Confidentiality*

Strict confidentiality was maintained at all times. No identifying data will be included in the database so participants will remain anonymous.

### *Informed Consent*

Informed consent was obtained from all participants in their own language as part of the initial study.

### *Ethical Approval*

Ethical approval (Rec: 057/2002) was obtained from the research and ethics committees of the Faculty of Health Sciences (FHS) of Universities of Cape Town and Stellenbosch, South Africa and allows for sub- analyses to be done.

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## **PART B: LITERATURE REVIEW**

### **Background**

Globally, HIV and TB are dual epidemics that affect children, especially those living in Sub-Saharan Africa. HIV-infected children with TB infection are at risk of more rapid progression to TB disease, more severe TB disease and increased mortality compared to HIV-uninfected children.<sup>1-4</sup> Conversely, TB increases HIV viral replication and progression to AIDS.<sup>5</sup>

Diagnostic difficulties and poor reporting systems have made it difficult to accurately quantify the global burden of childhood TB.<sup>6</sup> A community based South African study showed that children less than 13 years of age contributed approximately 13.7% of the total TB disease burden with a calculated incidence of 408/100 000.<sup>7</sup> In children under 2 years, this incidence was even higher, approximately 600/100 000.<sup>8</sup>

Prevention of TB in children is an important goal. The rationale for preventative therapy is based on the principle that mycobacterial infection occurs in two stages: infection and progression to disease. Children infected with TB have a much higher risk of progression to TB disease compared to adults. The TB bacillus can remain dormant for years but children are at highest risk of progression to disease within the first two years after infection.<sup>9</sup> The risk of TB disease in HIV negative infants is 40-50% within the first year after *M. tuberculosis* infection. This risk decreases to 25%, less than 10% and 2% in children 1-2, 2-5 and more than 5 years of age.<sup>10</sup> This high risk of disease in children exposed to TB provides the rationale for the international recommendations to make INH Preventative Therapy (IPT) available to children under 5 years with a household with TB contact.<sup>11 12</sup>

Data from immune-competent people has shown that IPT is effective for preventing progression from TB infection to disease. A Cochrane review investigated the effect of 6 to 12 month courses of IPT in HIV negative adults and children with TB exposure.<sup>13</sup> Eleven trials involving 73,375 patients were included. IPT decreased the risk of developing TB by 60% (95% CI 0.31-0.52). IPT reduced TB deaths (although this was not statistically significant) but not all cause mortality. The effect of IPT lasted at least 2 years following completion of IPT. The duration of treatment (6 months or 12 months) did not alter the

effect of IPT. Less than 1% of individuals developed hepatotoxicity while receiving IPT. However, only 6 studies included children. Effect estimates presented in the review were not stratified by age and thus the magnitude of the effect of IPT in children is unclear. Of the six paediatric trials included Comstock reported a risk reduction of 67% in children less than 14 years<sup>14</sup> and Ferebee and Mount of 50% in children less than 1 year.<sup>15</sup>

IPT in children was shown to work in other large trials some of which were not included in the Cochrane review as they were not randomized. Hsu conducted a prospective study spanning 30 years and enrolling 1882 children in the USA.<sup>16 17</sup> Children were given INH for 12-18 months and observed for a mean follow up time of 6 years. In contrast to previous natural history data, no child infected before 4 years of age developed disease.<sup>16 17</sup> This study and other large United States Public Health service trials formed the basis of the recommendations for the provision of IPT to all children less than 5 years of age when exposed to a TB contact.<sup>18</sup>

Children in high HIV and TB prevalence settings experience TB exposure at a young age because TB is common in HIV-infected women of childbearing age. A South African study from KwaZulu Natal showed that TB incidence in HIV -infected women at the time of delivery was increased tenfold compared to HIV negative women.<sup>19</sup> A study from India found that 3.5% of HIV-infected women that had recently given birth developed TB, with the median time to development of active TB disease being 3 months postpartum.<sup>20</sup> This study also described a much higher probability of death in infants whose mothers developed TB compared with infants whose mothers did not have TB.<sup>20</sup>

HIV-infected infants who have failed a Prevention of Mother to Child Transmission (PMTCT) program or who have not had access to PMTCT are at increased risk of progressing to TB disease after infection with *M. tuberculosis*.<sup>4</sup> A South African study reported that HIV-infected infants have a 20-fold higher risk of developing culture confirmed tuberculosis disease compared with HIV-uninfected infants.<sup>21</sup>

The large burden of TB in HIV-infected children can be prevented if available, effective strategies are implemented. The choice of prevention strategy depends on TB prevalence, HIV prevalence and available financial and human resources.

In HIV-infected children strategies such as Bacille Calmette-Guerin (BCG) vaccination, isoniazid preventive therapy (IPT) and antiretroviral therapy (ART) have been employed to prevent TB disease. World Health Organization (WHO) guidelines have recently been revised to recommend against BCG vaccination in HIV-infected infants due to the high risk of disseminated BCG and associated mortality.<sup>22</sup> IPT and ART prevent TB disease through different mechanisms but may be used in combination to reduce TB. Adult data has shown that IPT significantly decreased the risk of TB in HIV-infected adults taking ART in a high incidence operational setting,<sup>23 24</sup> but there is little information on the efficacy of IPT and ART for prevention of TB disease in HIV-infected children.

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## **Aim**

The aim of the review was to assess the evidence for IPT and ART as strategies to decrease TB disease in HIV- infected children.

## **Methods**

A literature search for publications on TB prevention in HIV-infected children was performed using the terms “HIV infection”, “tuberculosis”, “isoniazid preventive therapy”, “IPT”, “Antiretroviral therapy”, “HIV”, “AIDS”, “control”, “prevention”, “children”, “infants” and “paediatrics”. Medline and EmBase were searched for published studies from 1990 until December 2010. The International AIDS society website and the International Union Against Tuberculosis and Lung Disease website were searched for abstracts. The World Health Organization (WHO) site was searched for guidelines and publications on TB prophylaxis in HIV-infected children. Reference lists of identified primary studies, reviews and editorials were scanned. Studies that reported on mycobacteria other than tuberculosis were excluded. Editorials, case studies and case reports were excluded.

## **Results**

### **INH Preventative Therapy**

Data in HIV-infected adults have shown IPT is effective for preventing TB in individuals not receiving ART.<sup>25</sup> A recent Cochrane review including 12 randomized placebo controlled trials with a total of 8578 participants showed that IPT decreased the incidence of active TB in HIV-infected adults by 32% (RR 0.68, 95% CI 0.54 to 0.85).<sup>25</sup> All regimens regardless of drug type, frequency or duration of treatment showed similar efficacy however, multi-drug regimens were more likely to be discontinued due to adverse events than INH monotherapy. The protective effect of preventive therapy was more pronounced in individuals with a positive tuberculin skin test. Chemoprophylaxis did not reduce all cause mortality (RR 0.94, 95%CI 0.85 to 1.05).

#### *Paediatric Studies*

One systematic review<sup>26</sup> and two randomized controlled trials were found.<sup>27 28</sup>

A Cochrane review investigating the impact of IPT on TB disease and death in HIV-infected children found only one randomized controlled trial.<sup>26</sup> The study included in the Cochrane review was conducted in Cape Town which has one of the highest incidence rates of childhood TB in the world<sup>7</sup>. This study by Zar et al reported a 72% (HR 0.28, 95%CI 0.10 to 0.77) reduction in TB risk and a 54% (HR 0.46, 95% CI 0.22 to 0.94) decrease of all cause mortality in HIV-infected children receiving IPT.<sup>27</sup> Most children were severely immune compromised and very few were on ART at the time of the study.<sup>27</sup>

In contrast, results from the P1041 trial conducted in a similar setting in South Africa showed no effect of IPT on the risk of TB disease or all cause mortality.<sup>28</sup> The different results might be partly explained by differences in study populations (table 1). All children enrolled in the P1041 trial were infants (median age 3.3 months) and received ART at the time of enrolment, while the median age of children in the Zar study was 29 months and only 8% were on ART at enrolment. Children in P1041 were also less immune compromised compared to those enrolled in the Zar study. None of the children in the P1041 trial had TB disease before enrolment, while 16% of children had been treated for previous TB in the Zar study.



Incidence of side effects and adverse events was low in both studies. Zar et al reported grade 3 or 4 toxicity (as measured by a 10 fold increase in ALT from baseline) in five children (3%) receiving IPT and 8 children (6.1%) receiving placebo.<sup>27</sup> No neurological or cutaneous toxicity was observed. A study investigating toxicity associated with IPT in HIV-infected children on ART reported no episodes of acute liver failure and jaundice and a prevalence of grade 3 or 4 liver toxicity of 3.4%.<sup>29</sup> Safety data may also be extrapolated from larger adult trials. A study of 24221 adult HIV-infected mineworkers receiving IPT reported adverse events in only 0.54% of the cohort.<sup>30</sup>

Adherence to IPT in children enrolled on Zar study was reported as excellent with most children achieving adherence of more than 90%.<sup>31</sup>

### **Antiretroviral Therapy**

ART is a well established strategy to decrease the incidence of TB disease in HIV-infected adults.<sup>32</sup> A meta-analysis of nine observational cohort studies enrolling 37879 adult patients showed that use of ART was associated with a 67% (95% CI 61-73%; range, 54-92%) reduction in TB incidence.<sup>33</sup>

Data are limited in children. Besides age, immune status is the most important determinant of a child's risk of TB disease. A study from Cote d'Ivoire showed that the risk of TB was four times greater in children with a CD4% less than 15% than in those children with a CD4% of more than 15%.<sup>34</sup> In vitro studies have shown that HIV-infected children have an impaired ability to control mycobacterial growth compared with immune competent children.<sup>35</sup> Initiation of ART leads to a rapid and sustained reconstitution of specific anti-mycobacterial immune responses.<sup>36</sup> By improving immunity ART results in more effective containment of TB infection.<sup>36 37</sup>

### ***Paediatric Studies***

190 articles were identified and these abstracts were read. 12 were reviewed in full text. Five studies were not suitable: 2 reported on *non tuberculous mycobacteria*, 2 did not include children, 1 did not report on the impact of ART on TB risk. A total of 7 publications were eligible for inclusion (table 2). All but one study were from sub-Saharan Africa with a

high TB incidence; one study was from Jamaica with a TB incidence of 6.6 per 100,000 population.<sup>38</sup>

Of the seven studies, 3 did not indicate how the diagnosis of TB was made.<sup>39 40</sup> Two studies from the Democratic Republic of Congo (DRC) and Kenya had no access to sputum culture so used clinical scoring systems for TB diagnosis.<sup>41 42</sup> The study from the DRC had no access to HIV PCR for confirmation of HIV status in children under 18 months.

Most of the studies included older children between ages 12 months and 16 years, with the median age ranging from 4-7 years. ART regimens were not standardized. Most regimens consisted of two nucleoside analogues and a protease inhibitor or a non nucleoside reverse transcriptase inhibitor. The studies in DRC, Ivory Coast, Jamaica and South Africa were carried out in hospital clinics in urban settings. The Kenyan study took place in rural and urban clinics.

#### *Impact of ART on TB risk*

All studies reported a protective effect of ART on TB risk. The effect estimates ranged from 0.12 in South Africa to 0.51 in the DRC. One of the South African studies conducted a sensitivity analysis including only culture confirmed TB cases.<sup>43</sup> This did not change the effect estimate (0.30 compared to 0.25). The median TB risk reduction was 0.22. The magnitude of the effect is comparable with data from adults showing a TB risk reduction of 0.23 due to ART.<sup>33</sup> The studies included in this review were heterogenic with regards to study population and TB diagnosis. However effect estimates were remarkably similar with the exception of one study conducted in the DRC reporting a risk reduction of 0.51. Only one study was conducted in infants. The study was the only randomized controlled trial and thus is unlikely to be influenced by survival bias as some of the studies in older children might have been. Infants were randomly allocated to receive ART immediately or 3-6 month after HIV diagnosis. The trial found a 0.41 reduced risk for TB.<sup>44</sup>

## **Discussion**

### **INH Preventative Therapy**

The available evidence indicates that IPT decreases the risk of TB disease in immunosuppressed HIV- infected children and ART decreases TB risk in all HIV-infected children including infants. No effect for IPT in infants who were put onto ART early in life was shown.

The two studies investigating the protective effect of IPT in HIV-infected South African children have informed WHO's newest recommendations for HIV-infected children older than one year of age to receive IPT for 6 months irrespective of TB exposure or tuberculin skin test (TST). IPT may be continued for up to 3 years in areas of high TB prevalence. Children less than one year of age who have been started on ART for more than 3 months and are continuously screened for TB exposure should not receive IPT unless they have a documented TB exposure.<sup>45</sup>

These recommendations depend on well established and functional programs in high prevalence HIV and TB settings to regularly, reliably screen for and diagnose HIV and TB in infants. However, access to HIV PCR and TB culture may be limited in resource poor settings. Furthermore, diagnosis of TB is often sub-optimal and dependent on scoring systems which are particularly unreliable in HIV-infected children.<sup>46 47</sup> To safely implement IPT one needs to be able to reliably exclude TB disease thereby avoiding the emergence of drug resistant TB by the inadvertent use of mono-therapy.

Scale up of IPT for HIV-infected children may be challenging from a programmatic point of view. Evidence suggests that in HIV- uninfected children IPT is poorly implemented at a primary health care level. A recent survey in an urban community in South Africa evaluating the current system of IPT delivery showed that only 21% of children eligible for IPT received this. There were no standardized IPT management tools in place.<sup>48</sup> Another study from South Africa reported missed opportunities in 50% of children with TB contacts.<sup>21</sup> A study done in Malawi showed less than 10% of child TB contacts were screened for TB or offered IPT.<sup>49 50</sup> This stresses the need for implementing systems or strengthening existing systems to ensure scale up of IPT according to existing recommendations.<sup>48</sup>

Barriers to IPT implementation could possibly be overcome by simplified algorithms for TB screening, the creation of a national policy regarding IPT and engagement of affected communities.<sup>51</sup>

According to current recommendations, HIV-infected *infants* on ART should not receive IPT, with the proviso that they are regularly screened for TB exposure. However, in a high TB prevalence context such as South Africa, this is the group with the highest risk. One study reported a relative risk of culture confirmed TB of 24.2 in HIV-infected infants.<sup>21</sup> Moreover, regular screening of children for TB exposure and contact tracing of children exposed to an adult TB contact is notoriously poorly done within TB programs. Apart from trial conditions, vigilance is likely to be poor and tracing of contacts and provision of post exposure IPT not widely implemented.

The optimal duration of IPT in children has not yet been established. Studies from Botswana and South Africa involving HIV-infected adults suggest that increased benefits may be gained with a 36 month regimen as compared to a six month regimen.<sup>52 53</sup> This was not confirmed in a trial carried out in India.<sup>54</sup>

In summary, there is an urgent need for more data on IPT in HIV-infected children. Increasing numbers of children will be started on ART as access and affordability improves and the question of whether IPT provides additional benefits to ART in preventing TB disease is crucial.

### **Antiretroviral Therapy**

WHO now recommends starting ART as soon as possible in infants after a confirmatory HIV test at 6 weeks of age, irrespective of CD4 measures.<sup>55</sup> Roll out of ART to all HIV-infected infants presents several challenges. Diagnosis of HIV in infants relies on access to HIV PCR, which is lacking in most low income countries. Most importantly well functioning PMTCT programs are paramount to early diagnosis of HIV in children. While PMTCT programs have been scaled up over the last years gaps in access are still evident. ART programs for children are subject to operational problems such as continuous drug supply, good adherence and retention in care.<sup>56-58</sup>

An important finding is that although ART decreased the incidence of TB, HIV-infected children on ART are still at a higher risk of developing TB disease than HIV negative immune competent children.<sup>59</sup> Therefore other strategies are needed together with ART to decrease the risk of TB in these children. No studies have reported on the combined effect of ART and IPT in children.

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## **Conclusion**

The evidence suggests that IPT and ART independently decrease TB risk in HIV- infected children. The effect of IPT is inconclusive with the two trials showing conflicting results, probably as a result of different patient populations. IPT is protective in HIV-infected children older than 1 year who are not receiving ART. ART is protective in children of all ages but HIV- infected children are still at increased risk of TB disease compared to immune competent children. The impact of the combined effect of IPT and ART in HIV- infected children is unknown.

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**Tables.**

**Table 1: Differences between study population in Zar<sup>27</sup> and the P1041 study<sup>28</sup>**

Study	ZAR et al	P1041
Median age in months	25 (9-52)	3.3(3.1 -4)
CDC – B or C	88%	8%
Previous TB	16%	0% (exclusion criteria)
Median CD4 %	20 (14-28)	28 (6-58)
Received ART	9%	72%
Follow up time in months	5.7	8.3

**Table 2. Studies reporting tuberculosis incidence rates during in children antiretroviral therapy**

<b><u>Study</u></b>	<b><u>Setting</u></b>	<b><u>N</u></b>	<b><u>Study Period</u></b>	<b><u>Study Design</u></b>	<b><u>Med baseline CD4 count</u></b>	<b><u>TB incidence per 100 PY not on ART</u></b>	<b><u>TB incidence per 100 PY on ART</u></b>	<b><u>Adjusted Hazard Ratio (AHR)</u></b>	<b><u>National TB Incidence per 100000 population</u></b>
Walters et al, 2008 <sup>59</sup>	South Africa	290	January 2003 to December 2005	Retrospective folder review	Most had severe CD4 count depletion	53.3 (9 months preceding ART)	6.4 (during 13.5 months on ART)	0.12 (AHR)	+/- 900
Edmonds et al, 2009 <sup>41</sup>	Democratic Republic of Congo	364	Dec 2004 to April 2008	Observational cohort	48.3% severe immune depletion	20.4	10.2	0.51	430
Kouakoussui et al, 2004 <sup>40</sup>	Ivory Coast	270	No start date to Dec 2003	Observational cohort	8.7%	8.5	1.9	0.22	606
Martinson et al, 2009 <sup>43</sup>	South Africa	1132	September 1994 to June 2006	Retrospective cohort	15%	21.1	6.4	0.3	+/- 900
Pierre et al 2008 <sup>39</sup>	Jamaica	197	Sept 2002 to August 2005	Prospective cohort	595 (absolute)	1.07	0.14	0.13	6.6
Braitstein et al 2009 <sup>42</sup>	Western Kenya	6535	Dec 2001 to Jan 2007	Retrospective observational	Not specified for overall cohort	7.2	22.2	0.32 (IRR) 0.15 (AHR)	305
Violari et al, 2008 <sup>44</sup>	South Africa	377	August 2005 to June 2007	Randomized controlled trial	35.2%	20.2 (19/125)	8.3(17/252)	0.41	+/- 900



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## **PART C**

**The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis (TB) in HIV-infected children in a high TB incidence setting.**

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### **Contribution of the candidate**

The candidate undertook the study concept and supervised and aided in acquisition of data, analysis and interpretation of data. She drafted the article and the final version incorporating comments from co authors.

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**The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis (TB) in HIV- infected children in a high TB incidence setting.**

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**What is the key question?**

Does INH prophylaxis (IPT) reduce TB in HIV-infected children on antiretroviral therapy (ART)?

**What is the bottom line?**

IPT provides additional protection against TB disease in children taking ART

**Why read on?**

To examine how to safely reduce TB using IPT and ART in combination

## **Abstract**

**Background:** Tuberculosis (TB) is a major cause of morbidity and mortality among HIV-infected children. Strategies to prevent TB in children include isoniazid preventive therapy (IPT) and antiretroviral therapy (ART). IPT and ART have been reported to reduce TB incidence in adults but there are few studies in children.

**Objective:** To investigate the combined effect of IPT and ART on TB risk in HIV-infected children

**Methods:** A cohort analysis was done within a prospective, double-blinded, placebo-controlled trial of INH compared to placebo in HIV-infected children in Cape Town, South Africa, a high TB incidence setting. In May 2004 the placebo arm was terminated and all children were switched to INH. ART was not widely available at the start of the study, but children were started on ART following the establishment of the national ART program in 2004. Data were analyzed using Cox proportional hazard regression.

**Results:** After adjusting for age, nutritional status and immunodeficiency at enrolment INH alone, ART alone and INH combined with ART reduced the risk of TB disease by 0.22 (95% CI 0.09-0.53), 0.32 (95% CI 0.07-1.55) and 0.11 (95% CI 0.04-0.32) respectively. INH reduced the risk of TB disease in children on ART by 0.23 (95% CI 0.05-1.00).

**Conclusions:** The finding that IPT may offer additional protection in children on ART has significant public health implications, as this offers a possible strategy for reducing TB in HIV-infected children. Widespread use of this strategy will however require screening of children for active TB disease.

Trial registration - Clinical Trials NCT00330304

## Introduction

Tuberculosis (TB) is an important cause of morbidity and mortality in children living in high TB incidence settings.<sup>1 2</sup> The incidence and severity of childhood TB is increased by HIV infection. A recent study reported a relative risk for developing culture proven TB in HIV-infected infants, of 24.2 (95% confidence interval [CI] 17-34) compared to HIV-uninfected infants.<sup>3</sup> Furthermore, HIV-infected children with TB infection are at risk of more rapid progression to disease, more severe disease and increased mortality.<sup>4-6</sup>

Bacille Calmette Guérin (BCG) vaccination, isoniazid preventive therapy (IPT) and antiretroviral therapy (ART) are possible strategies to prevent TB in HIV-infected children. World Health Organization (WHO) guidelines have recently been revised to recommend against BCG vaccination in HIV-infected infants due to the high risk of disseminated BCG and associated mortality.<sup>7</sup>

Limited data suggest that IPT<sup>8</sup> or ART<sup>9 10</sup> can independently reduce TB incidence and mortality in HIV-infected children. We have previously reported that IPT (irrespective of TB exposure) reduced the risk of TB in HIV-infected children not on ART by approximately 50%.<sup>8 11</sup> Another South African study found that IPT did not decrease risk of TB in HIV-infected children under 12 months.<sup>12</sup> These studies have informed the recent revised WHO guidelines recommending IPT for 6 months for all HIV-infected children above 12 months of age.<sup>13</sup>

A retrospective study found that ART substantially reduced TB incidence in HIV-infected children, with 53 TB cases per 100 patient years prior to use of ART compared to 6.4 on ART.<sup>9</sup> However, HIV-infected children on ART still have a higher risk of TB than HIV-uninfected children.<sup>9</sup>

Observational studies suggest that INH preventive therapy is protective in adults receiving ART.<sup>14 15</sup> Several randomized trials investigating the combined effect of IPT and ART are underway in adults but data in children are scarce. The objective of this study was to investigate the combined effect of IPT and ART on TB risk in HIV-infected children living in a high TB incidence setting.



## Methods

A cohort study was performed as part of a prospective, double-blinded, placebo-controlled trial of INH, given with cotrimoxazole either daily or three times a week in HIV-infected children in two centers in Cape Town, South Africa. The study area has one of the highest incidence rates of childhood TB globally, 407/100 000 in children less than two years of age.<sup>16</sup> The study started in January 2003; in May 2004 the placebo arm was terminated on the recommendation of the Data Safety and Monitoring Board (DSMB) as interim analyses demonstrated significant benefit of INH on mortality, the primary outcome. INH was found to reduce mortality by 54% [0.22- 0.95 95% CI].<sup>8</sup> Thereafter, all children were placed on INH daily or three times a week until December 2007 when INH was discontinued on the advice of the DSMB as almost all of the children had started on ART. A cohort analysis was performed to investigate the combined effect of INH and ART on TB risk in children enrolled in the study from January 2003 through December 2007.

### *Study population*

The study population comprised HIV-infected children older than 8 weeks attending Red Cross Children's Hospital, University of Cape Town or Tygerberg Children's Hospital, Stellenbosch University. 98% of children were black South African from low socio-economic background. Inclusion criteria were a weight greater than 2.5kg, and ability to attend regular follow-up visits. Exclusion criteria were current use or need for IPT, prior hypersensitivity to sulphur drugs or INH, severe anaemia (haemoglobin < 70 g /l), neutropaenia (absolute neutrophil count < 400 cells /ul) and non-reversible renal failure. TB disease at the time of enrolment was not an exclusion criterion but children completed TB therapy according to national guidelines and thereafter were randomized to INH or placebo. All children were classified according to WHO clinical staging (stage 1 to 4).

### *Follow-up*

The study team evaluated the children every four weeks for the first six months, every six weeks for the second six months and thereafter every two to three months depending on medical and social circumstances. Clinical and historical data were collected at each visit. A tuberculin skin test (TST, 2 TU RT23, Statens Serum Institute, Copenhagen, Denmark), and chest radiograph were done at 6 monthly intervals. A child exposed to a TB source (case), hospitalized for respiratory illness, or developing symptoms of TB at any time during the

study period was investigated for TB infection and disease. For pulmonary TB this included a TST, a chest radiograph and two induced sputum specimens for acid fast staining and *Mycobacterium tuberculosis* (*M. tuberculosis*) culture. Additional specimens were submitted as clinically indicated. Children exposed to a household TB case were given IPT according to standard guidelines and then returned to their initial randomization without being un-blinded.

### *INH*

Enrolment began in January 2003. Children enrolled before May 2004 were randomly allocated to receive INH (10 mg/kg) or placebo. All children receiving placebo were switched to INH in May 2004. All children enrolled thereafter received INH. INH was stopped in all children in December 2007.

### *ART*

ART was not widely available at the start of the study but was obtained for some children through participation in pharmaceutical trials or charitable donations. Children were started on a double NRTI backbone (Zidovudine, Lamivudine, Stavudine) combined with either a NNRTI (Efavirenz or Nevirapine) or a protease inhibitor (Ritonavir/Lopinovir or Ritonavir only). Standard guidelines for ART in children were developed after the establishment of the national ART program in 2004. Thereafter increasing numbers of children initiated therapy according to medical and social criteria described in the national guidelines.

### *Diagnosis of TB*

Cases of TB were classified as definite or probable according to clinical, radiological and microbiological findings as follows: (1) Definite TB: culture positive for *M tuberculosis* on a sputum or other sample; (2) Probable TB: chest radiograph suggestive of TB (lymphadenopathy, miliary pattern, pleural effusion, bronchial compression or parenchymal infiltrate) plus at least one of the following: a positive TST, a history of a close contact with an adult with TB, loss of weight or failure to gain weight within the previous 3 months, or a positive smear microscopy for acid fast bacilli on sputum. Chest radiographs were reported by a single radiologist, blinded to the diagnosis of TB, according to a standardized format. Probable cases were reviewed by an independent experienced pediatrician blinded as to whether the child was on INH, placebo and/or ART.

### *Study design*

A prospective cohort study was performed to investigate the effect of INH and ART on TB incidence. Individuals were censored at death, date of TB diagnosis, loss to follow-up or on the 31 December 2007. Individuals could contribute time to four groups: time on placebo only, time on INH only, time on ART only, time on ART and INH (figure 1).

### *Confounding variables*

The degree of immunodeficiency at enrolment was grouped according to WHO CD4 classification.<sup>17</sup> Categories 1 and 2 (insignificant or mild) and categories 3 and 4 (moderate or severe) were grouped together. WHO clinical staging at enrolment was grouped as stage 1 and 2 or stage 3 and 4 combined. The z-scores for nutrition were grouped as  $>-2$  (normal or mild) or  $<-2$  (moderate or severe).

### *Statistical analysis*

All analysis was conducted using Stata (version 10.1, College Station, Texas). Descriptive statistics of the baseline data and follow-up data of the cohort were calculated as medians, 25<sup>th</sup> and 75<sup>th</sup> percentiles and frequencies.

Cox proportional hazards regression was performed using time since randomization as the time scale. Records were split for time dependent exposure and age at follow-up. The analysis was performed taking dependency of records into account. Univariable analyses were conducted using immunodeficiency, anthropometric z-scores and age at enrolment (grouped as 0-2 years, 2-5 years and more than 5 years), gender, prior TB, daily or three times per week regimens for INH /cotrimoxazole, placebo, INH, ART and INH and ART.

Hazard proportionality was assessed by analysis of scaled Schoenfeld residuals.

Age at follow-up and immunodeficiency at enrolment were included a priori in the multivariable model. Possible other confounders including WHO clinical staging and nutritional status at enrolment and prior TB, gender were assessed with regards to their impact on the effect estimates. Variables acting as confounders were included in the final model.

#### *Ethical approval*

The study was approved by the ethics committees of the Faculty of Health Sciences, University of Cape Town and of Stellenbosch University. Written informed consent was obtained from a parent or legal guardian.

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## Results

### *Baseline characteristics*

Two hundred and ninety eight children (median age 2.09 years) were enrolled. At enrolment 117 children were randomly assigned to receive placebo and 181 to INH (figure 1). Of 117 children assigned to receive placebo, 78 were switched to INH after May 2004 while 39 were censored (lost to follow-up, died or diagnosed with TB) before May 2004. In summary there were 3 INH groups: (I) children who received INH throughout follow-up (n=181), (II) children who initially received placebo and were then switched to INH (n=78) and (III) children who only received placebo during follow-up (n=39). Overall, 39 (13%) children were on ART at enrolment, while 135 (45%) started ART during the study. The total time of follow-up was 495.65 person years: 42.02 person years on placebo/no ART, 12.67 person years on placebo/ART, 207.73 person years on INH/no ART and 233.23 person years on INH/ART. A total number of 37 children died: 15 on placebo, 2 on ART, 14 died on INH and 6 on INH plus ART.

At enrolment, most children were mildly undernourished with a median height for age z-score of -1.91 and weight for age z-score of -1.34 (table 1). The majority had severe clinical disease, with 82% in WHO stage 3 or 4. Most (83%) had advanced or severe immunodeficiency (table 1). Forty-one (13%) children were diagnosed with TB at enrolment. The median follow-up time was 21.7 months with a median time on INH of 20.3 months and median time on ART of 17.9 months. During this period, 39 cases of TB were diagnosed (19 definite and 20 probable). INH was well tolerated with excellent adherence using pill count and adherence counseling. There was no increased risk of elevated liver enzymes, jaundice or fulminant liver failure in children taking ART and IPT.<sup>18 19</sup>

### *Effect of INH, ART and INH plus ART*

Univariable Cox regression analysis showed no association between gender, weight for age z-score, weight for height z-score and history of TB disease (table 2). More advanced clinical disease and immunodeficiency at enrolment increased the risk of TB disease, as did more severe stunting. ART alone and INH alone reduced the risk of TB disease by 0.35 and 0.24 respectively. INH and ART combined reduced TB risk by 0.14 (95% confidence interval (CI) 0.05-0.35) compared to placebo.

Multivariable analysis showed that, after adjusting for age at follow-up, nutritional status and immunodeficiency at enrolment INH alone reduced the risk of TB by 0.22 (95% CI

0.09-0.53). ART alone reduced TB risk by 0.32 (95% CI 0.07-1.55) compared to placebo. The combination of INH and ART reduced the risk of TB by 0.11 (95% CI 0.04-0.32) (table 2). Restricting the analysis to children receiving ART revealed a TB risk reduction of 0.23 (95% CI 0.05-1.00) comparing INH versus no INH.

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## Discussion

This is the first study to suggest that IPT reduces TB risk in children receiving ART. Overall, there was a 0.11 risk reduction of TB disease in children receiving both INH and ART compared with children receiving neither. While, INH and ART independently reduced TB risk by 0.22 and 0.32 respectively, the combination of INH and ART reduced the risk by 0.11. In children on ART INH reduced the risk of TB disease by 0.23.

The protective effect of ART on TB risk has been reported in children.<sup>9 10</sup> ART reduces susceptibility to *M tuberculosis* by improving immunity, and enabling more effective containment of TB infection.<sup>20 21</sup> The benefit of IPT in preventing TB disease in TST positive HIV-infected adults has clearly been shown.<sup>22</sup> Studies in HIV-infected adults found a TB risk reduction of 76% in individuals receiving IPT and ART compared with those receiving neither.<sup>14 15</sup> A possible explanation for this added benefit is the effect of INH in treating latent infection and preventing progression to disease. In young children, TB disease usually represents progression of primary infection rather than reactivation of latent infection, therefore the mechanism of the additional effect of IPT in children receiving ART as occurred in our study may be through containment of primary infection.

Diagnosis of TB infection or pulmonary disease is challenging in HIV-infected children due to anergy and difficulty in obtaining a microbiological diagnosis. A concern for implementing IPT is inadvertent use in pulmonary TB, thereby promoting drug resistance. In this study all children were carefully screened for TB at enrolment, including repeated induced sputum samples. As evidence of the effectiveness of screening, 41 (13.8%) children were diagnosed with TB at enrolment and commenced on TB therapy. This high proportion of newly diagnosed TB through active case finding highlights the importance of appropriate screening tools before the rolling out of IPT.

Intensive surveillance for TB infection and disease was done in the study through close clinical, radiological, immunological and microbiological follow-up. We did not identify TB in any of the children who died. Three children developed INH-resistant TB although one had contact with a multi drug resistant case. A study including children from this cohort reported 3.4% of grade 3 and 4 elevation in ALT in children on ART and INH. All children were asymptomatic and no children developed clinical jaundice or fulminant hepatic failure.<sup>18</sup> Age, nutritional status and CD4 count at enrolment were confounders and as such included in the final multivariable model. The association between age and TB did not reach significance which is not unexpected as unlike immuno-competent children in whom the risk

of developing severe TB disease is highest in infants; HIV-infected children are at risk of *M tuberculosis* disease at all ages. The level of immunodeficiency was not significantly associated with TB in this cohort. Most children had moderate or severe immunosuppression at enrolment but CD4 percentage is unlikely to correlate with CD4 percentage during follow up, as use of ART during the study would affect CD4 measures. The strengths of this study include blinding to eliminate diagnostic bias and follow-up of long duration that ensured reliable outcome ascertainment. A limitation of this study was that ART was not randomly allocated. The early termination of the placebo arm reduced the follow-up time that this group could contribute, and may have reduced the power but continuation of the placebo arm was not ethically possible, given the effect of INH on mortality and the recommendation by the DSMB.<sup>8</sup>

Survival bias might have potentially influenced the results. When the data were analyzed using a nested matched case control design, where 1 case was matched on age and follow-up time to 1-4 controls results were similar to the cohort study. Changes in TB incidence for example, a decrease in TB incidence, could also have biased the estimates. National and local TB incidence was increasing during the study period.<sup>23</sup> Finally, the protective effect of ART is not immediate and may be seen many months after initiating treatment. The study was done over 5 years with a median follow-up time of 20 months, thus enabling sufficient time to investigate the effect of ART.

Initiation of ART may unmask TB, which could dilute the possible protective effect of ART on TB risk. Due to the young age group, latent TB infection is not common and thus immune reconstitution inflammatory syndrome (IRIS) due to unmasking would be unlikely. In addition, no cases of BCG IRIS were documented. Currently IPT is not recommended for HIV-infected children less than 12 months (without history of TB exposure).<sup>13</sup> If IPT, ART and Cotrimoxazole prophylaxis were to be initiated as a 'package of care' at two to three months of age, then the need to screen for active TB would be greatly reduced and IRIS would be less likely.

The finding that IPT is protective in children on ART has significant public health implications, as this offers a possible strategy for reducing TB in HIV-infected children. The effect of IPT is obviously dependant on the level of TB exposure and the results of this study should be interpreted in a context of a high TB prevalence setting. They may not apply to a low TB prevalence setting. Widespread use of this strategy will however require screening of children for active TB disease. The optimal duration of IPT has not been established, but long term use as in this study appears effective and safe.



## **Conclusion**

This is the first study to suggest a protective effect of INH in children on ART. The expansion of IPT for HIV-infected children has a number of health system challenges, including the ability to detect and exclude active TB disease before commencing IPT. Nevertheless, IPT may safely reduce TB incidence in HIV-infected children on ART. Further studies are needed to determine the optimal duration that IPT can be safely continued in HIV-infected children who are continuously exposed to TB in high incidence settings.

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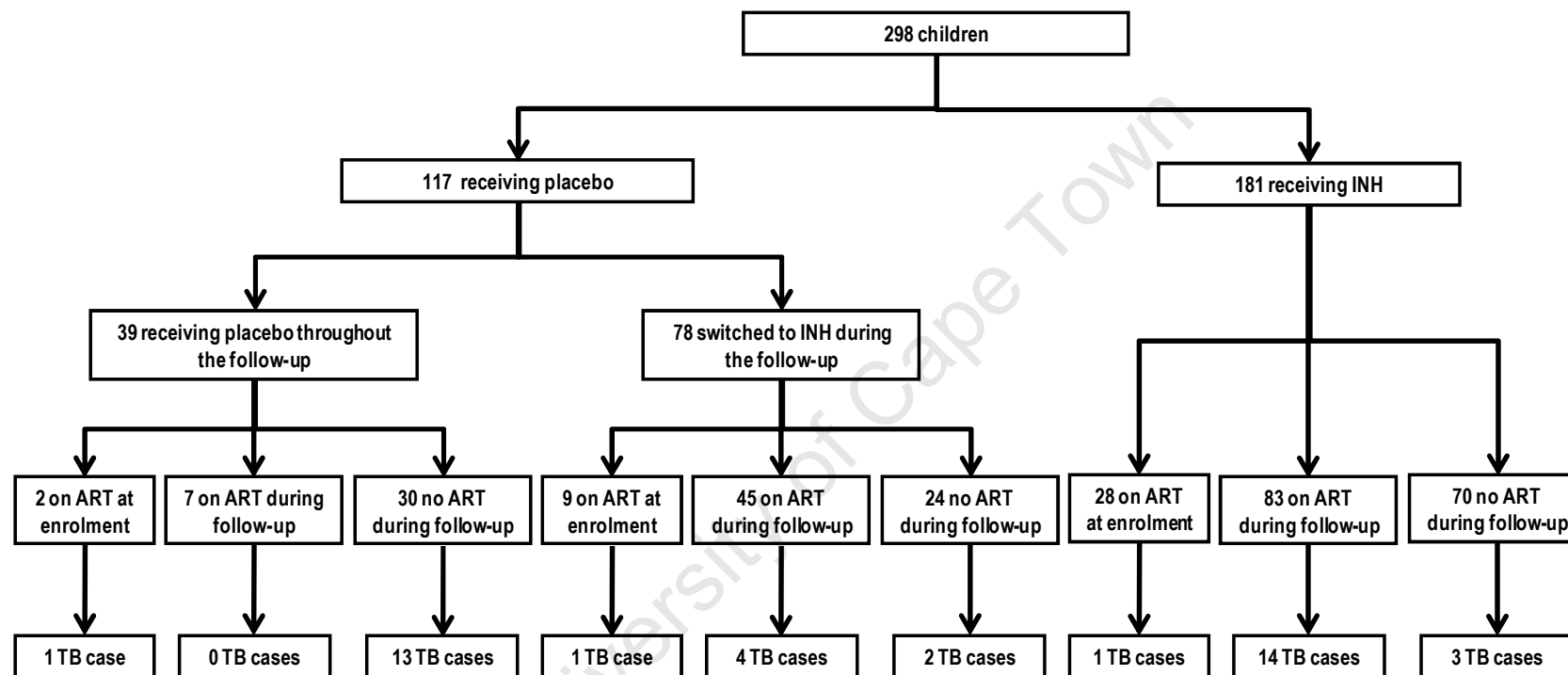
**Table 1: Baseline characteristics of children enrolled (N=298)**

Variables		N (%) or Median (IQR)
Age (years)		2.09 (1.03, 4.11)
Gender	Female	135 (45.3%)
	Male	163 (54.7%)
WHO clinical stage at enrolment	Stage 1	12 (4.1%)
	Stage 2	41 (14.1%)
	Stage 3	177 (60.8%)
	Stage 4	61 (21.0%)
WHO CD4 depletion at enrolment	Category 1 = not significant	30 (10.1%)
	Category 2 =mild	20 (6.7%)
	Category 3 = advanced	49 (16.4)
	Category 4 = severe	199 (66.8%)
Anthropometry at enrolment	Height for age Z- score	-1.91 (-2.87, -0.94)
	Weight for age Z-score	-1.34 (-2.41, -0.43)
	Weight for Height Z-score	-0.17 (-1.07, 0.72)
History of TB disease	None	236 (79.2%)
	Prior to enrolment	21 (7.0%)
	TB treatment at enrolment	41 (13.8%)
Cotrimoxazole/INH	Daily	145 (48.7%)
	3 times a week	153 (51.3%)
INH	Placebo throughout the study	39 (13.1%)
	Initial Placebo, switched to INH	78 (26.2%)
	INH throughout the study	181 (60.7%)
ART	At enrolment	39 (13.1%)
	Started during the study	135 (45.3%)
	No ART	124 (41.6%)
Time on ART (months) N=174		17.92 (10.6; 26.2)
Time on INH (months) N=259		20.03 (14.5; 27.8)
Follow up time (months) N=298		21.7 (9.5, 27.4)

**Table 2: Effect of INH, ART and INH combined with ART on TB risk**

Variables		Univariable		Multivariable	
		HR (95% CI)	P	HR (95% CI)	P
Gender	Male	1			
	Female	1.01 (0.54; 1.90)	0.970		
Age at follow-up	0-2	1			
	2-5	0.78 (0.37; 1.66)	0.511	0.74 (0.33; 1.55)	0.445
	>5	0.68 (0.29; 1.60)	0.384	0.79 (0.33; 1.91)	0.603
WHO clinical stage at enrolment	1 or 2	1			
	3 or 4	1.52 (0.60; 3.86)	0.390		
Immunodeficiency at enrolment	Mild	1			
	Advanced/severe	2.44 (0.73; 8.09)	0.144	2.74 (0.78; 9.68)	0.117
Z-score (height for age) at enrolment	Mild	1			
	Moderate/severe	1.80 (0.96; 3.39)	0.068	1.84 (0.99; 3.42)	0.055
Z-score (weight for age) at enrolment	Mild	1			
	Moderate/severe	1.00 (0.51; 1.96)	0.098		
Z-score (weight for height) at enrolment	Mild	1			
	Moderate/severe	1.20 (0.40; 3.57)	0.760		
History of TB disease	No	1			
	Yes	0.83 (0.38; 1.80)	0.625		
Cotrimoxazole/INH	Daily	1			
	3 times a week	0.89 (0.48; 1.67)	0.714		
Placebo		1			
ART		0.35 (0.07-1.63)	0.179	0.32 (0.07; 1.55)	0.157
INH		0.24 (0.11-0.55)	0.001	0.22 (0.09; 0.53)	0.001
INH and ART		0.14 (0.05-0.35)	<0.001	0.11 (0.04;0.32)	<00.01

Figure 1: Children included in the study



Follow-up time:

Placebo, no ART: 42.02 person years  
 Placebo and ART: 12.67 person years  
 INH, no ART: 207.73 person years  
 INH and ART: 233.23 person years

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## **PART D**

### **Appendix 1- Consent and Information forms**

IYunivesithi yaseStellenbosch – iCandelo lobuNzululwazi ngezeMpilo  
2002C/073

ULWAZI NGEZIGULANE NEFOMU YEMVUME  
9 Oktoba 2004

*Ubuchule bokukhusela izifo zephanyazo ezosulelayo kubantwana abanentsholongwane iHIV boMzantsi Afrika: Ukuthelekiswa koluhlu lwemithetho yempilo emibini i- trimethoprim-sulphamethoxazole (cotrimoxazole) prophylaxis ehamba okanye engahambi ne-isoniazid – luba nempembelelo ekuguleni, ukufa, intsholongwane nokuhlaselwa sisifo sePhepha (TB).okutshintshileyo:ukubhalisa kwabantwana abatsha abafumana amayeza entshlongwana kagawulayo (Haart ) kwicandelo le-INH okanye iplasibho.*

Wena nomtwana wakho niyacelwa ukuba nithathe inxaxheba kufundo ngophando lonyango olwenziwa eRed Cross, kunye nesibhedlela sabantwana iTygerberg. ISebe lobuNzululwazi ngaBantwana neZifo zabo neMpilo yaBantwana kwiYunivesithi yaseStellenbosch neYunivesithi yaseKapa ziqhuba olu fundo. Olu lwazi lulandelayo luya kuchaza olu fundo kunye nendima yomntwana wakho njengomtu othatha inxaxheba. Nceda ufunde oku ngononophelo, ungabuza nemibuzo ukuba awuqinisekanga. Olu fundo luya kuqhutywa ngokuhambelana nezikhokhelo ze-Declaration of Helsinki ne-MRC ne-ICH (International Committee of Harmonization).

#### **Imvelaphi**

Umntwana wakho usengozini yokuqalwa sisifo esosulelayo sesifuba esibangelwa yintsholongwane ebizwa ngokuba yi-*Pneumocystis carinii*. Esi sifo esosulelayo sikwenza ubenesifuba esibuhlungu (pneumonia). Xa ufuna ukuzikhusela kufuneka umntwana asele iyeza elibizwa ngokuba yi-**cotrimoxazole** (okanye Cozole / Bactrim) kathathu ngeveki okanye yonke imihla.Eliyeza liyakunikwa kuphela ukuba umntwana kufuneka elisebenzisile.Ukuba izinga leseli zegazi ziphakamile yaye umntwana zange abenayo inyumoniya ngaphambili akuyo mfuneko ukunika eliyeza okanye ipilisi.Ngugqirha oyakugqiba ngoko. Umntwana wakho ukwasengozini yokufumana isifo sephepha (TB) nesifumaneka lula kummandla waseKapa ngoba umntwana wakho akakwazi ukusilwa esi sifo ngenxa yentsholongwane kaGawulayo (HIV). Ukuzikhusela kwisifo sephepha kufuneka unikwe iyeza elibizwa ngokuba yi-**isoniazid (INH)**.

Kubalulekile ukuqwalasela ukuba esisifundo siqale ngo Januwari 2003. Ibhodi ejongene nokhuseleko lwamayeza esifundo liye laqwalasela iziphumo ngesifundo saza safumanisa ukuba i-INH inoncedo ebantwaneni. Ngeloxesha bekukho abantwana abayi-278 ababhalisiweyo .Impembelelo ekufeni ilicala. Ukususela ngoko bonke abantwana abakwi-plasibho banikezwa i-INH.Bonke abantwana abafayo babengafumani mayeza entsholongwana (HAART).Lamayeza abonisiile kuphando ukuba abalulekile ekugcineni abantwana bephilile

Version 3.0  
29 September 2004

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2002C/073

**PATIENT INFORMATION AND CONSENT FORM**

May 23rd, 2003

*Strategies for prevention of opportunistic infections in HIV-infected South African children: Comparison of 2 trimethoprim-sulphamethoxazole (cotrimoxazole) prophylaxis regimens with and without concomitant isoniazid – impact on morbidity, mortality, bacterial resistance and incidence of tuberculosis.*

You and your child are requested to participate in a medical research study that is being done at Red Cross and Tygerberg Children's hospitals. The Department of Paediatrics and Child Health of the Universities of Cape Town and Stellenbosch are conducting this study. The following information will describe the study and your child's role as a participant. Please read this carefully and feel free to ask any questions. The study will be conducted according to the Declaration of Helsinki and to MRC and ICH (international committee of harmonization) guidelines.

**Background**

Your child is at risk for developing a chest infection caused by a germ called *Pneumocystis carinii*. This infection may cause a severe chest infection (pneumonia). To prevent this, a medicine called **cotrimoxazole** (or Cozole / Bactrim) should be taken by your child either 3 times a week or everyday. Your child is also at risk for getting tuberculosis (TB) because this is so common in the Cape Town area and because your child is unable to fight off infections very well because of his/her HIV disease. To prevent TB, a medicine called **isoniazid (INH)** may be given.

**Purpose of the study**

The aims of this study are to

- (1) Compare how effective giving cotrimoxazole 3 times a week is with giving it everyday for preventing chest and other infections.
- (2) Investigate whether giving INH either 3 times a week or everyday can prevent TB.

**Procedures in the study**

Your child will be given cotrimoxazole to take either 3 times a week or everyday. Your child will also be given INH as a tablet or a pill that looks like the INH tablet but does not contain INH (placebo) to take either 3 times a week or everyday. Your child will be seen every month at the Infectious Diseases clinic to check how he/ she is doing. At the first visit to the clinic, a blood test will be done to measure your child's ability to fight infection (immune status) and a blood specimen will be stored to measure the amount of HIV virus in your child. A swab from the nose will also be taken and sent to the laboratory where tests will be done to identify the germs living in his/her nose. A nose swab will be done every 6 months on you child to see whether cotrimoxazole or INH change the type of germs in your child's nose. A

## Appendix 2: Data capture form for each study visit

TMP-SMX/INH Study														
Study No: <input type="text"/>					Follow Up Visits					<div style="display: flex; justify-content: space-around;"> <span>Site</span> <span>RXH</span> <span>TBH</span> </div>				
Date Completed: d <input type="text"/> m <input type="text"/> y <input type="text"/>										By Whom: <input type="text"/>				
Follow-up visit (months): <input type="text"/> 3 <input type="text"/> 6 <input type="text"/> 9 <input type="text"/> 12 <input type="text"/> 15 <input type="text"/> 18 <input type="text"/> 21 <input type="text"/> 24 <input type="text"/> 27 <input type="text"/> 30 <input type="text"/> Other: <input type="text"/>														
Surname: <input type="text"/>										Name: <input type="text"/>				
Date of birth: d <input type="text"/> m <input type="text"/> y <input type="text"/>														
Hospital/Clinic: <input type="text"/>										FN <input type="text"/>				
Address, if changed: <input type="text"/>														
Change in caregiver: <input type="text"/> Y <input type="text"/> N <input type="text"/>										If yes, name: <input type="text"/>				
Tel number(s): <input type="text"/>														
Another contact no. <input type="text"/>														
<b>History since last visit</b>														
										Duration in days				
Cough: <input type="text"/> Y <input type="text"/> N <input type="text"/>										RTHC present : <input type="text"/> Y <input type="text"/> N <input type="text"/>				
LOW or RFT: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Fever: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Diarrhoea: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Vomiting: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Runny nose: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Wheeze: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Poor feeding: <input type="text"/> Y <input type="text"/> N <input type="text"/>														
Other: Specify <input type="text"/>														
<input type="text"/>														
<input type="text"/>										<input type="text"/>				
<input type="text"/>										<input type="text"/>				
<input type="text"/>										<input type="text"/>				
<input type="text"/>										<input type="text"/>				

1

### Appendix 3: TB data capture form

TMP-SMX/INH Study																
Study No: <input type="text"/>										Site: <input type="text"/> RXH <input type="text"/> TBH <input type="text"/>						
TB Information																
Hospital ID: <input type="text"/>																
Group: <input type="text"/> Daily <input type="text"/> MWF <input type="text"/>										If # before 17/05/04, indicate <input type="text"/> INH <input type="text"/> Placebo <input type="text"/>						
<b>Section 1: TB prophylaxis completed prior to # (as on Enrolment form)</b>																
TB Px prior to #		<input type="text"/>	<input type="text"/>	<input type="text"/>	If yes how many courses? (Please complete a section for each course.)										<input type="text"/>	<input type="text"/>
<b>Course 1:</b>																
Details available		<input type="text"/>	<input type="text"/>	<input type="text"/>	Incomplete		Date Px started		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
Place started:		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
TB Contact details		Name:				Relationship:										
		MDR-TB				Suspected				Unknown						
Mantoux done		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
Prophylaxis used		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
Completed Prophylaxis		<input type="text"/>				<input type="text"/>				<input type="text"/>						
Date Completed: <input type="text"/> By whom: <input type="text"/>																
<b>Course 2:</b>																
Details available		<input type="text"/>	<input type="text"/>	<input type="text"/>	Incomplete		Date Px started		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
Place started:		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
TB Contact details		Name:				Relationship:										
		MDR-TB				Suspected				Unknown						
Mantoux done		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
Prophylaxis used		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
Completed Prophylaxis		<input type="text"/>				<input type="text"/>				<input type="text"/>						
Date Completed: <input type="text"/> By whom: <input type="text"/>																

#### **Appendix 4 : letter of approval of protocol**

Dear Dr Lisa Jane Frigati

Candidature Approval ( FRGLIS001 )

Degree

MMed in Paediatrics

Title

The impact of isoniazid prophylaxis and antiretroviral therapy on tuberculosis (TB) incidence in HIV- infected children in a high TB incidence setting.

Department

Paediatrics

Supervisor

Prof H. Zar

Ethics Approval

057/2002

I am pleased to advise that the Chair of the Dissertations Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained by publication in the Dean's Circular, Med06/2010.

Kind Regards

Nomkitha Nyamende(Ms)

Postgraduate Admin

Faculty of Health Sciences

University of Cape Town

Medical School

Tel: 021 406 6751

Fax: 021 406 6584

E-mail:n.nyamende@uct.ac.za

## **Appendix 5: Ethics Approval**



UNIVERSITY OF CAPE TOWN

Research Ethics Committee  
Faculty of Health Science  
E46- 26 Old Main Building, Groote Schuur Hospital,  
Observatory, 7925  
Queries : Xolile Fula  
Tel : (021) 406-6492 Fax: 406-6411  
E-mail : Xfula@curie.uct.ac.za

08 April 2002

**REC REF: 057/2002**

Dr. H. Zar  
Paediatrics

Dear Dr. Zar

**STRATEGIES FOR PREVENTION OF OPPORTUNISTIC INFECTIONS IN HIV-INFECTED SOUTH AFRICAN CHILDREN: COMPARISON OF 2 TRIMETHOPRIM-SULPHAMETHOXAZOLE PROPHYLAXIS REGIMENS WITH AND WITHOUT CONCOMITANT ISONIAZIDE-IMPACT ON MORBIDITY, MORTALITY, BACTERIAL RESISTANCE AND INCIDENCE OF TUBERCULOSIS**

Thank you for submitting your study to the Research Ethics Committee for review.

*It is a pleasure to inform you that the Committee has formally approved your study however the investigator/s must inform patients and family members that antiretroviral therapy is available either in trials or can be purchased privately at their own expense.*

*Attached is a copy of members who attended the meeting.*

**Please quote the above Rec. reference number in all correspondence**

Yours sincerely

Signature removed

**A/PROF. CR.SWANEPOEL**  
**CHAIRPERSON**